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14. ABSTRACT The purpose of this project is to investigate molecular events of the preclinical stages of mammary cancer, specifically, the intersection between the development of genome demethylation, retrotransposon transcriptional activity, and retrotransposon-driven transcription of cellular genes in an engineered mouse model of mammary cancer. During the last 12 months collection of material for planned molecular analyses and preparation of RNA and DNA for molecular library construction was completed. Construction of two replicates of the planned RNA libraries was completed in Australia, a third replicate was unable to be completed due to RNA degradation in some samples. The data was forwarded to my collaborator Dr Edwards at Washington University in St Louis, who has initiated library analyses. DNA was also forwarded to Dr Edwards who has commenced construction of DNA libraries with preliminary analyses expected by the end of 2014 or early in 2015. A laboratory technician (Tim Smith) has been accepted as a PhD student at the University of Adelaide, and his project will be to complete the wet lab molecular analyses and verification of <i>in silico</i> results with independent samples. We believe that the eventual findings will provide insights into understanding the role of genome hypomethylation and expression of retrotransposons in cancer ontogeny, and may impact cancer prevention in the future.					
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1. Introduction

This project is designed to address the subject of mammary cancer development. The purpose of the project is to investigate molecular events occurring in the preclinical stages of mammary cancer; the results may lead to insights into cancer prevention in the future. Specifically, the project investigates the intersection between genome demethylation, retrotransposon transcriptional activity, and retrotransposon-driven transcription of cellular genes. Retrotransposon promoters are well recognized to function as alternative promoters for different cellular genes, generating chimeric transcripts that may or may not function in the same way as transcripts from the regular gene promoter. Transcriptional activation of retrotransposons is strongly linked with their CpG DNA methylation, and global genomic demethylation is one of the commonest molecular changes in malignancies. The project tests the hypothesis that, in preclinical stages of tumour development, progressive genomic demethylation leads to increased transcriptional activity of retrotransposons and this, in turn, leads to transcription of otherwise silent genes, potentially setting up molecular conditions that favour cancer development. We developed a genetically engineered mouse model in which a specific mammary cell population is fluorescently marked upon initial transcriptional activation of the SV40 large T antigen (SV40Tag) oncogene. SV40Tag is transcriptionally activated during pregnancy and lactation, and the mice are predisposed to develop mammary cancer after 3 pregnancies and lactations. Using this model, populations of marked cells can be collected for integrated analysis of gene expression, promoter usage, and DNA methylation after defined amounts of exposure to SV40Tag during different stages of preclinical cancer development.

2. Keywords

Cancer
Cancer development
Mammary cancer
Mouse model
Retrotransposon
Alternative promoters
DNA methylation
SV40 large T antigen
Methyl-MAPS

3. Overall Project Summary

Since the previous report, the laboratory technician employed in the first two years of the project, Tim Smith, has been granted an Australian Postgraduate Award to pursue a doctoral program continuing his work on this project. Planned work completed during the reporting period includes completion of animal work, RNA and DNA isolation, and construction of deeply sequenced paired-end RNA libraries. DNA samples and the RNA

library data for analysis has been forwarded to my collaborator Dr John Edwards at Washington University in St Louis. RNA library analyses are poised to begin in his laboratory, and DNA processing for deep sequencing and Methyl-MAPS analysis is underway.

Problems encountered during this period included the following:

- i. Loss of funding for the laboratory assistant at the end of the previous reporting period; this was resolved by Australian Postgraduate Award to Tim Smith to support a structured PhD program with research focusing on analysis and validation of library data from this project.
- ii. Intermittent problems with RNA isolation occurred, resulting in lower than anticipated RNA yields from mammary tissue. The problems were ascribed to the technical difficulties of having to work in different locations for each of the several steps of the isolation procedure. Technical advances in minimizing the quantity of RNA required enabled construction of expression libraries for two of the planned three biological replicates, while keeping RNA samples in reserve for quantitative real-time PCR verification of expression library analyses.
- iii. Delivery of the final tranche of funds for this project was delayed for reasons beyond our control; however we applied for and were granted a 4 month further no-cost extension, and submitted a revised Statement of Work to that effect (modified contract W81XWH-11-1-0401 P00002).

4. Key Research Accomplishments

Nothing to report.

5. Conclusion

In its third year, the project has moved ahead with the planned molecular analyses. We believe that the experimental replicates can be analysed as planned and the results at least partially tested on independent material in the next 4 months. Until that time no scientific conclusions can be made from this work.

6. Publications, Abstracts, and Presentations

- Smith T., Edwards J., Peaston AE. Mammary Cancer and the Activation of Transposable Elements. Poster presentation at Australian Society for Medical Research, SA Division, Annual Scientific Meeting April 2014.

7. Inventions, Patents and Licenses

8. Reportable Outcomes

- Australian Postgraduate Award received by Tim Smith for work continuing this project
- Poster presentation at Australian Society for Medical Research, SA Division, Annual Scientific Meeting April 2014 (see above)
- Manuscript in preparation (Tim Smith), Genome Methylation, Retrotransposition-driven Gene Expression And Mammary Cancer, a Review.

9. Other Achievements N/A

10. References N/A

11. Appendices N/A